STUDIES ON THE RELATION BETWEEN AMITOSIS AND MITOSIS.

II. Development of the Testes and Spermatogenesis in Moniezia.

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The material for this study was obtained from the same species, *viz.*, *Moniezia expansa* and *Moniezia planissima*, as that of the first paper of this series[1] and most of it, in fact from the same chains. The methods of fixation, observation and record are the same as those already described in that paper. Various details in the spermatogenesis are only briefly considered, since they are not directly connected with the chief purpose of the paper.

I. *The Formation of the Testes.*

The testes develop from cells of the parenchyma which do not differ visibly from other cells of the same region. They appear in the dorsal region of the central parenchyma. Before their appearance two kinds of cells are visible in the parenchyma: one of these is smaller and surrounded by more or less cytoplasm apparently without definite boundary and usually elongated in the dorso-ventral direction, with one or more fibrillar extensions at each end. Figs. 1, *A–D* (Pl. VII.), show parenchymal cells of this kind in the earliest stages of testis formation. Amitotic division of the nucleus is occurring in each case. Fig. 1, *A*, shows a case in which the two parts of the dividing nucleus stain differently Fig. 1, *C*, a case of the endogenous form of nuclear division which was described in the preceding paper, while Figs. 1, *B*, and 1, *D*, show late stages in division, the one by constriction, the other by formation of a nuclear plate. For a description of these and other forms and stages of amitosis in *Moniezia* the reader is referred to the preceding paper of this series.

The other form of cell existing in the parenchyma before testis

[1] Child, "Studies on the Relation between Amitosis and Mitosis I. Development of the Ovary and Oögenesis in Moniezia," Biol. Bull., Vol. XII., No. 2, 1907.

formation is much larger, and its cytoplasm is highly vacuolated and more distinctly bounded from the parenchymal matrix about it. These cells appear always to be connected with deeply staining fibers which extend dorso-ventrally across the central region of the proglottid and which I take to be dorso-ventral muscle fibers since they and the cells connected with them are similar to those of the muscular layers. Fig. 2, *A* (Pl. VII.), shows one of these cells with a portion of its fiber. The fiber passes directly through the cell-body on one side of the nucleus and the cytoplasm extends visibly for a considerable distance along the fiber. The evidence that these cells develop into testes is very strong. Fig. 2, *B* (Pl. VII.), represents a section through nucleus and body of one of these cells in which the nucleus is apparently undergoing amitosis. Whether this particular case would have developed into a testis it is of course impossible to determine. But Figs. 2, *C*, and 2, *D* (Pl. VII.), represent characteristic cases slightly more advanced. Here several nuclei are contained in a space which corresponds closely with the form of the muscle cell and contains what seem to be strands of the old vacuolated cytoplasm, while about some of the nuclei a layer of more deeply staining cytoplasm is visible, apparently in process of formation. Through the space which apparently represents the region previously occupied by the body of the muscle cell passes the fiber. The presence of the fiber and the well marked outline of the space seem to me to constitute very strong evidence in favor of the conclusion that each of these groups of nuclei have arisen by the division of a nucleus of a muscle cell. That these groups develop into testes there can be no doubt. Their development can be followed from proglottid to proglottid without the slightest difficulty, and there are no other similar groups of nuclei in the parenchyma. Fig. 2, *E* (Pl. VII.), shows a case in which the muscle fiber is apparently undergoing degeneration. In all of these cases amitotic division of the nuclei is taking place. The figures give only a few examples of the cases observed. In a brief account of the history of these cells already published additional figures are given.[1] If these observations are correct, and I have, so far as

[1] Child, "The Development of Germ Cells from Differentiated Somatic Cells in Moniezia." *Anat. Anz.*, Bd. XXIX., Nos. 21 and 22, 1906.

I am aware, taken all possible precautions to assure myself that they are, it seems impossible to escape the conclusion that in *Moniezia* the male germ-cells may develop from cells which have previously been differentiated and functional in the soma. Generalization from this conclusion is, however, no more justifiable than is that so often made from observations which seem to point in the opposite direction. It does not seem probable that uniformity exists here any more than in other features of development in regard to which premature generalizations mark like wrecks the dangers along the channel of biological thought.

In slightly later stages it is difficult or impossible to determine with certainty which testes have arisen from the smaller and which from the larger cells. Figs. 3, *A*–3, *F* (Pl. VII.), represent young testes which probably developed from the smaller parenchymal nuclei: amitosis is visible in all cases except Fig. 3, *F*, in which mitosis is occurring. In the hundreds of testes examined at this stage four cases of mitotic division have been observed of which one is shown in this figure. All of the observed cases were found in a single chain of *Moniezia expansa*, another fact which seems to indicate that the relative frequency of the two forms of division may vary. It was not possible to determine the number of chromosomes with certainty, but it was more than twelve, the number shown in the figure.

Figs. 4, *A*–4, *D* (Pl. VIII.), represent cases which probably developed from the large cells — muscle cells. Such stages are found in the same proglottids as the stages shown in Figs. 3, *A*–*F*, and are clearly larger and contain more nuclei than the latter. Fig. 4, *A*, shows a case in which a small nucleus, apparently not a part of the large mass, is also seemingly involved in the development. Cases of this sort are not infrequent, and the small nucleus often becomes one of the membrane-nuclei, though the latter appear in many cases to arise from the same primordium as the germ-cells themselves. Here, as in the development of the female organs, it is difficult to resist the impression that the development of these organs is the result of some localized stimulus or condition and that any cells within reach of this factor may become involved.

At this stage the nuclei of the developing testis lie in a con-

tinuous mass of cytoplasm, which is more or less distinctly marked off from the parenchymal substance, but still shows fibrous extensions into the parenchyma. The development of the testes differs from that of the ovary in that none of the parenchymal substance is included within the testis. This difference is merely the consequence of the fact that the testes usually develop from a single nucleus and its surrounding cytoplasm, while the ovary develops from a large number of the parenchymal nuclei.

II. *The Growth of the Testes Preceding the Spireme Stage.*

During the period preceding the appearance of the spireme in a part of the cells the development of the testes consists of increase in size as the result of numerous divisions, chiefly amitotic and of formation of the membrane about the testis and of the vasa efferentia.

The formation of the membrane occurs at an early stage from the cytoplasm of cells about the periphery of the proliferating mass. Fig. 5, *A* (Pl. VIII.), shows one of these cells with the membrane forming an extension of the cytoplasm. The vas efferens is formed in the same manner (Fig. 6, *A*, Pl. VIII.). The membrane-forming cells are few in number and the nuclei apparently undergo degeneration in later stages, for they are very rarely found in the fully developed testis.

The contents differ somewhat in appearance according to the method of fixation employed. After Hermann or chrom-oxalic the nuclei appear in most cases to be imbedded in a syncytial mass of cytoplasm, cell boundaries being indistinguishable or sometimes faintly visible, though cavities or vacuoles are frequently observed. After sublimate and some of the sublimate mixtures in all but the earlier stages the cytoplasm appears to have undergone shrinkage and to be more or less definitely concentrated about each of the nuclei. In later stages the individual cells appear more distinct after any fluids. The earliest stages of testis-development are certainly syncytial and without doubt the individualization of the cells takes place gradually. In consequence of the shrinkage of the delicate and probably highly fluid protoplasm caused by the sublimate fluids the distinctness of the cells is exaggerated. Most of the figures of these stages (Figs. 5,

A–6, *B*, Pl. VIII., 7, *A*–8, *B*, Pl. IX.) are taken from the chrom-oxalic preparations and cell-boundaries do not appear in most cases though they may be present. Fig. 9, *D* (Pl. IX.) is taken from a sublimate preparation at a rather late prespireme stage and shows the cells as distinct.

But for present purposes the nuclei are of chief importance. Figs. 5, *A*–5, *C* (Pl. VIII.) represent stages just after the formation of the membrane. In Figs. 5, *A*, and 5, *B*, amitoses are visible and in Figs. 5, *B*, and 5, *C*, two of the very infrequent cases of mitosis in these stages are shown. Figs. 6, *A*, and 6, *B* (Pl. VIII.) are from slightly later stages: Fig. 6, *A*, is a section through one side of a testis and does not show the full size. Both figures show amitoses and Fig. 6, *B*, shows one case of mitosis. Figs. 7, *A*, and 7, *B* (Pl. IX.), are from still later stages. In the latter figure one case of division of a nucleus into three parts is shown. The nuclei in which such divisions take place are usually larger than the others, often lie near the center of the testes and are similar in appearance to the large nuclei along the axis of the developing ovarian follicles which were mentioned in the preceding paper. In Figs. 8, *A*, and 8, *B* (Pl. IX.), stages just preceding the first appearance of the spireme, each with several amitoses, are shown. In Fig. 8, *A*, one of the large nuclei dividing into three parts is seen near the opening of the vas efferens.

In Fig. 9, *A*–9, *D* (Pl. IX.), cells or cell-groups from various stages containing amitoses of special interest are shown. Figs. 9, *A*, and 9, *C*, represent cases of the form of amitosis designated endogenous in the preceding paper in which the two nuclei resulting from division do not occupy the entire space within the old membrane. Numerous cases of this sort have been observed in the testes and have been examined with great care. Fig. 9, *B*, is a case of triple division and Fig. 9, *D*, a case of cytoplasmic division proceeding from within outward. This figure is from a sublimate preparation.

During these stages mitoses are rare. Very often not a single case is found in any of the numerous testes of a proglottid. In other proglottids a number of testes may show one or more each. In general the relative frequency of mitosis appears to vary in

different chains and in different proglottids. In one chain of *M. planissima* for example mitosis has been observed only very rarely though the chain has been carefully examined; in another it was found to be much more frequent. In all cases, however, amitosis is the predominant form of division in these stages.

III. *Formation of the Spireme and the Growth Period.*

In the stages before spireme-formation, or as it has often been called, synapsis, all the nuclei in the testes are similar in appearance and contain a large deeply staining nucleolus with perhaps a few smaller granules.

Suddenly a part of the nuclei begin to increase in size and a spireme appears (Figs. 10, *A*–13, *B*, Pl. X.). The formation of the spireme takes place in the manner described for the ovary in the preceding paper of this series. The change does not appear to begin in any particular region of the testis. Sometimes different groups of cells in different regions of the same testis give rise to a spireme while about them and between them lie others still unchanged and undergoing amitosis. From the first appearance of the spireme in the testes until the formation of spermatozoa is completed the multiplication of the spermatogonia which remain in the prespireme stage goes on, chiefly or wholly by amitosis and some of the cells thus produced are continually passing into the spireme stage. Consequently the stage is not characteristic of any particular period of development of the testis as a whole after its first appearance; in the older testes some groups of cells in the spireme stage and some groups of spermatogonia in prespireme stages are always to be found.

In some testes before the spireme stage appears and frequently afterward some of the cells are seen to be more or less pear-shaped in form with the pointed ends radially arranged about a center and united by strands of cytoplasm (Figs. 10, *A*, 11, *B*, Pl. X.). The number of cells in a group of this kind varies from three or four to eight or ten. All the cells of a group pass into the spireme stage simultaneously. Whether such groups are due to the persistence of cytoplasmic connections from previous divisions or to the formation of new connections it has been impossible to determine, but it seems possible from the varying size of

the groups that they are merely the result of connection of cells lying near each other.

The grouping of the cells is not by any means a characteristic feature in these stages. Frequently cells which are entirely separate from each other pass into the spireme stage simultaneously (Figs. 10, *B*, 11, *A*, Pl. X.). As will appear below, the grouping is merely the first step in a process characteristic of spermatogenesis and observation indicates that in some cells it begins before the spireme appears in others not until later.

As in the ovary the spireme is usually massed at one side of the nucleus as in many other cases of synapsis and is often visibly connected with the nucleolus (Figs. 12, *A*–12 *B*, Pl. X.), which, however, does not decrease in size but increases as the nucleus grows larger (Figs. 10, *A*–13, *B*, Pl. X.).

The appearance of the spireme is accompanied by an increase in the amount of cytoplasm. Comparison of the cells in this stage with those in earlier stages in Figs. 10, *A*, 10, *B*, and 11, A (Pl. X.), shows this difference clearly. The cytoplasm in the spireme stages also appears somewhat more dense in structure and stains a little more deeply. Here as in the ovary the apparent connection of the nuclear changes with the growth of the cytoplasm is most striking.

As is usual, this stage in the testes is not accompanied by such extreme growth of the cytoplasm as in the ovary nor by any formation of yolk, but is soon followed by the spermatogenetic divisions.

But the stages following the spireme are not the same in all cells ; the later development follows two very different lines. In the later stages of the spireme period it is possible to distinguish two different sorts of nuclei. In the one (Fig. 13, *A*, Pl. X.) the nucleolus has disappeared and the spireme is very dense and occupies almost the whole periphery of the nucleus. Careful examination and comparison has convinced me that these nuclei are in preparation for the first spermatocytic mitosis. The nuclei of the other sort are considerably larger (Fig. 13, *B*, Pl. X.), the spireme is much less dense and more irregular in form and does not occupy the whole periphery but is still massed more or less at one side and the nucleolus is still intact. I am confident that

these nuclei do not represent earlier stages than those in Fig. 13, *A*, for they are always larger than the latter and the irregularity of the spireme is not found in the earlier stages. These nuclei are the first stages in a remarkable process of fragmentation which will be described in a later section. Their relative frequency as compared with the others appears to vary in different chains, proglottids and regions. Sometimes they seem to be more, sometimes less numerous than the others.

(To be continued.)

Explanation of Plates.

Plate VII.

Fig. 1. *A–D*, testes forming from parenchymal cells: amitotic division in all cases.

Fig. 2. *A*, muscle cell; *B*, muscle cell undergoing amitosis; *C*, *D*, *E*, muscle cells developing into testes; in *E* the muscle fiber is apparently undergoing degeneration.

Fig. 3. *A–F*, young testes; in *F* a case of mitosis.

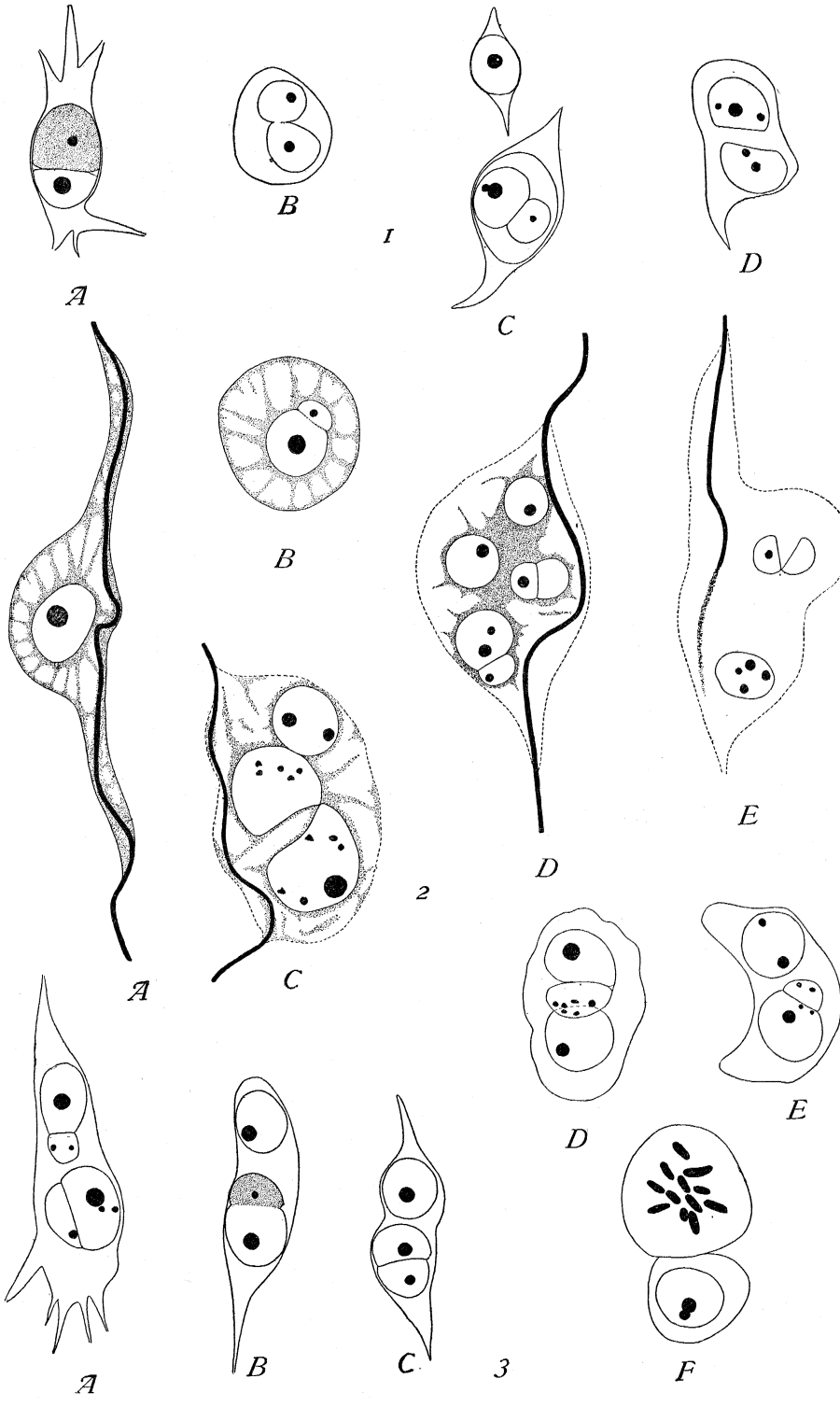
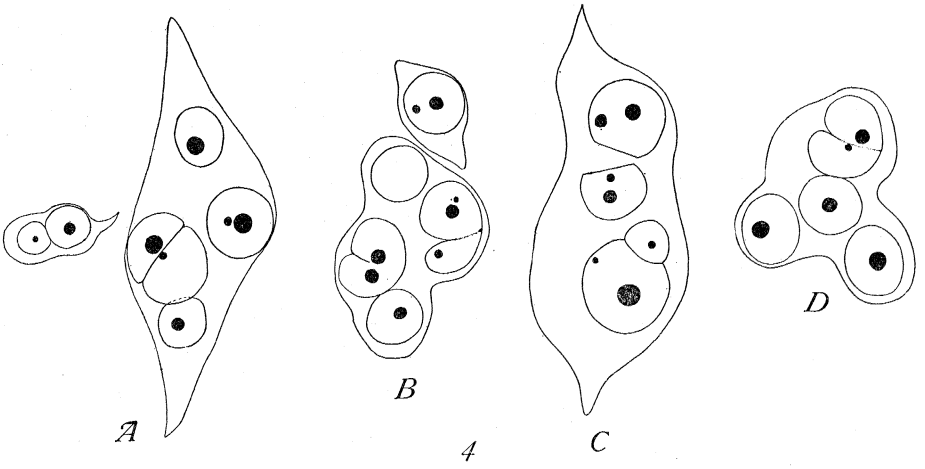

A
B
1
C
D
A
B
C
2
D
E
A
B
C
3
D
E
F

PLATE VIII.

FIG. 4. *A–D*, young testes showing amitosis.

FIG. 5. *A–C*, young testes after formation of membrane and vas efferens; *B* and *C* contain mitoses.

FIG. 6. *A*, *B*, developing testes.



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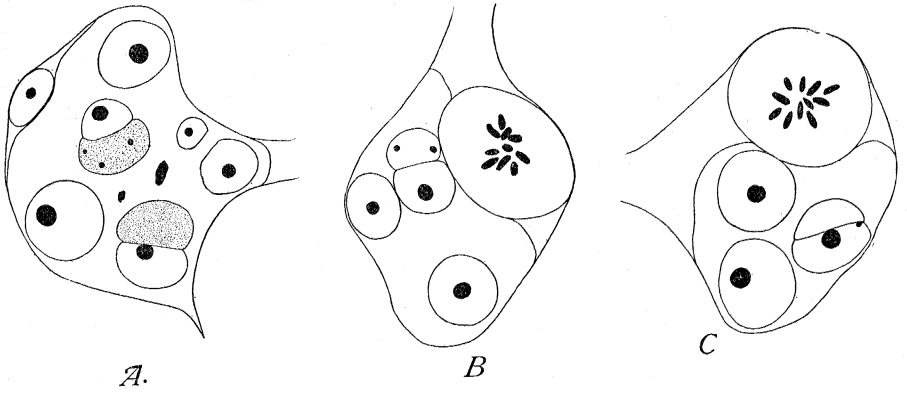

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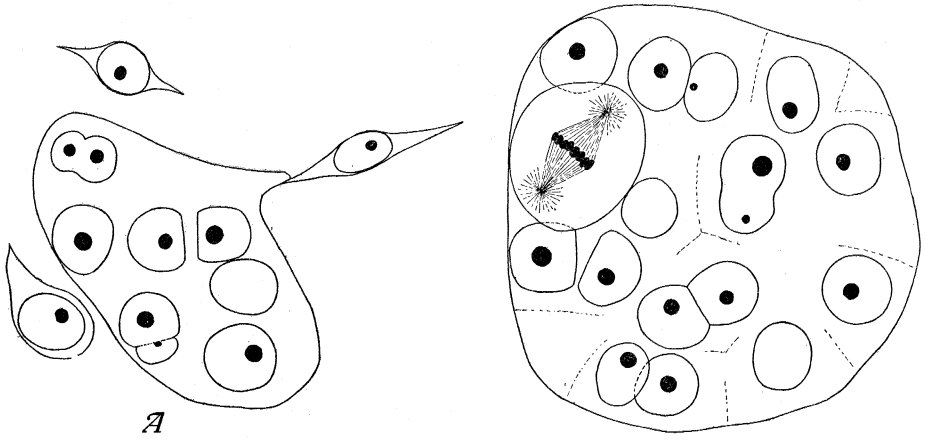

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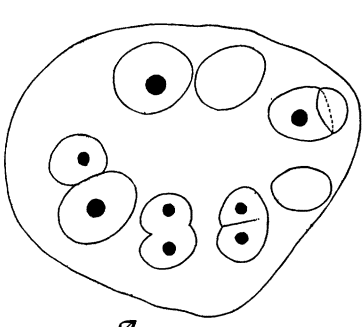
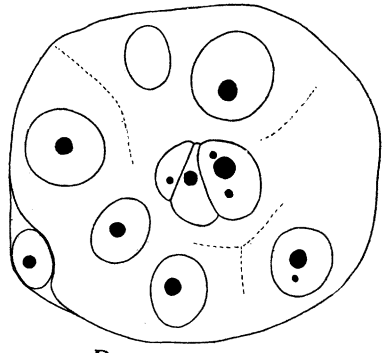
PLATE IX.

FIG. 7. *A*, *B*, developing testes.
FIG. 8. *A*, *B*, developing testes in later stages.
FIG. 9. *A*–*D*, amitoses from developing testes.



A

7



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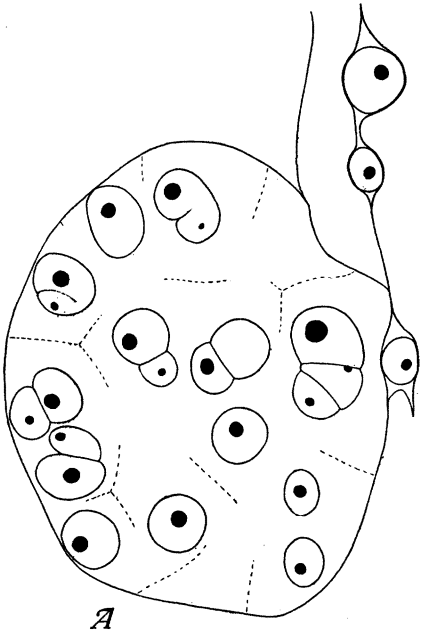
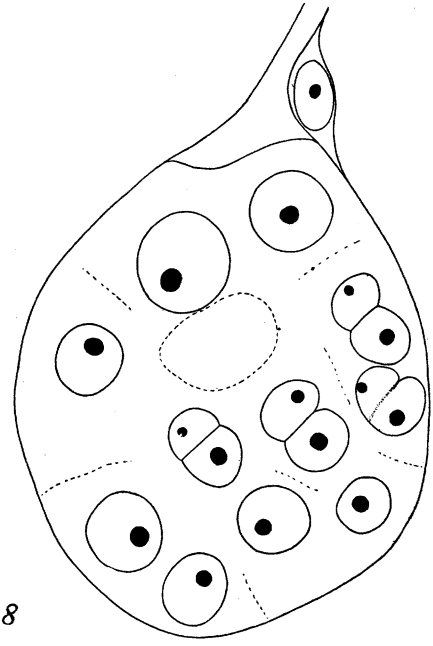


A

8



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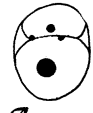
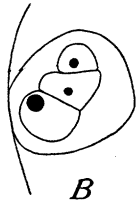


A



B

C

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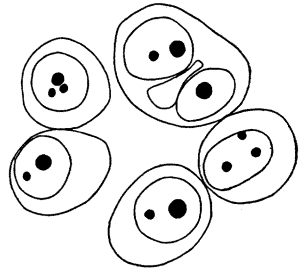


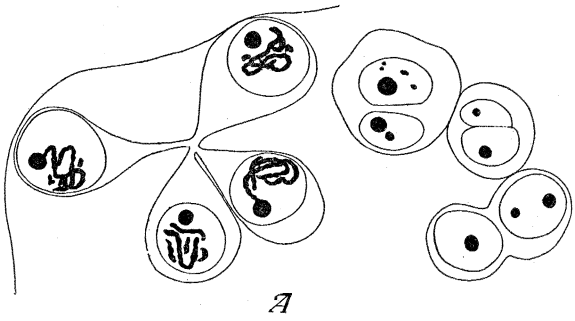
D

PLATE X.

FIG. 10. *A*, *B*, Fig. 11; *A*, *B*, the spireme stage. Figs. 10, *A*, and 11, B, show the first stages in fusion of the spermatocytes to form a cytophore.

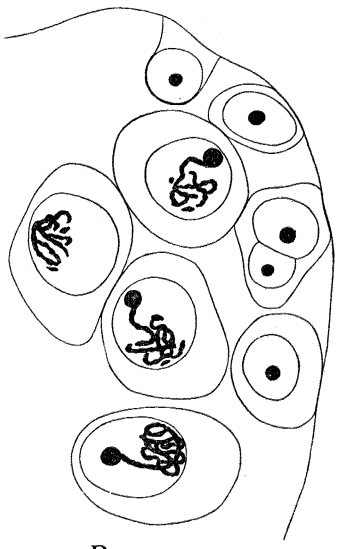
FIG. 12. *A*, *B*, spireme stages.

FIG. 13. *A*, preparation for first spermatocytic mitosis; *B*, preparation for fragmentation.



A

10



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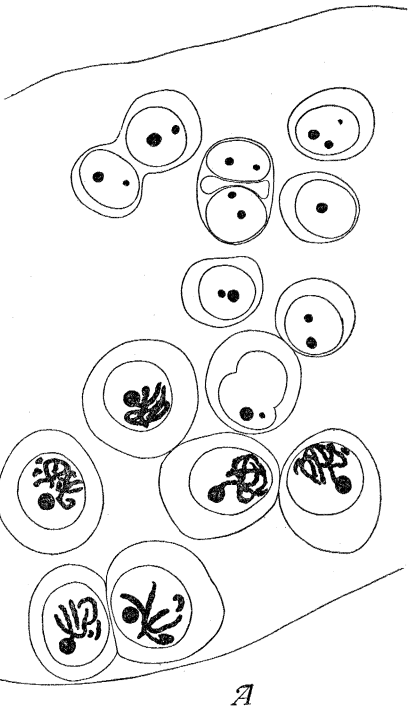
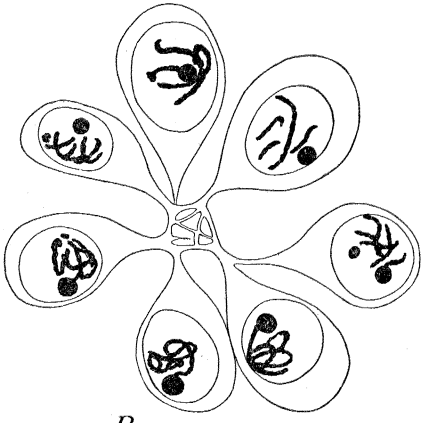


A

11



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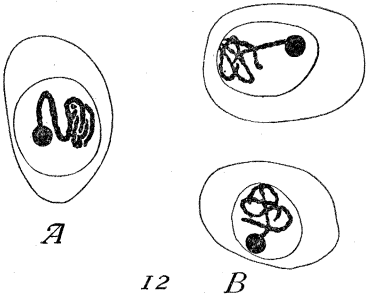
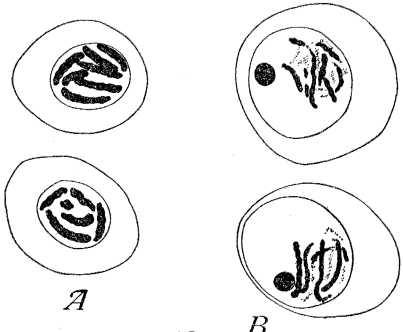


A B

12



A B

13